The Laboratory in the Diagnosis of Communicable Diseases

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MANY of the communicable diseases that formerly constituted a significant proportion of the medical practice of physicians have become all but extinct. Some of these, such as typhoid fever, are so infrequent as to be seen rarely, if at all, in the practice of most physicians. Numerous other communicable diseases occur so infrequently that the specific laboratory procedures available to assist the physician in the diagnosis of these conditions are not generally known. The rarity of these diseases makes their recognition by the physician all the more difficult. Moreover, only recently have specific laboratory procedures been developed to assist the physician in making a definitive diagnosis in a number of communicable diseases, particularly those caused by viral and rickettsial agents.

The cases reported in California during the past five years of certain of the communicable diseases in which specific laboratory procedures are available to assist the physician in diagnosis are given in Table 1. It will be noted that a number of these diseases are reported infrequently. We have no way of knowing how many diagnosed cases are not reported or how many cases occur that are not recognized. In addition, in some instances, such as "Q" fever, the diseases have not been made reportable and our only evidence of frequency of occurrence is acquired by specific epidemiological investigation. Several diseases of frequent occurrence are also listed because they still present practical problems in specific diagnosis in which the laboratory can be of assistance.

The following report deals with the laboratory only as it applies to the identification of the specific infectious agent either by isolation and study of the agent itself, or by the demonstration of the appearance of specific antibodies in the blood serum of the host. The commonly used clinical laboratory procedures, such as blood counts, are not considered in this report.

Several recent outbreaks of some of these communicable diseases have revealed a lack of understanding by the practicing physician of the bacteriological and serological laboratory aids that are available. In several instances there has been an undue delay in the diagnosis of diseases through failure to utilize these available services. Some of the more practical laboratory aids will be itemized herein. This is mostly a review of well-known procedures, but experience in the state laboratory has revealed the need for periodic restatement of the more important procedures. In addition, some of the newer procedures in the viral and rickettsial disease field will also be briefly mentioned, together with an indication as to how these services may be secured.

TABLE 1.—*Cases of Certain Diseases Reported in California During the Five Year Period 1943-1947.*

Diseases	Cases
Typhoid fever	879
Paratyphoid fever—Total	400
A	35
B	305
C	52*
Type unknown	8
Dysentery, bacillary	1,669
Brucellosis	1,391
Plague	3
Relapsing fever	60
Tularemia	36
Influenza	31,877
Infectious pneumonia	16,208†
Rocky Mountain spotted fever	9
Typhus fever	228
Q fever	178‡
Lymphogranuloma venereum	1,063
Psittacosis	26
Encephalitis:	
Total cases reported	828
Western equine (+ Lab.)	59
St. Louis (+ Lab.)	33
Mump encephalitis (+ Lab.)	9§

*Made reportable July 1, 1946.
†Including atypical and virus pneumonia.
‡Not reportable. Cases found in epidemiological investigations to March 1, 1948.
§Laboratory procedure started July 1, 1946.

TYPHOID AND OTHER ENTERIC FEVERS

Reference to Table 1 will indicate that typhoid fever has become an infrequent disease. During the past five years, only 879 cases have been recorded in California. Physicians may practice for years, or even a lifetime, without encountering a case of typhoid. This results in a low index of suspicion which in turn not infrequently results in a considerable delay in arriving at a diagnosis. In one recent outbreak, involving 21 cases, the onset of the first case was July 3, yet the first case was not diagnosed until July 25, some 22 days later.[2]

In typhoid fever there are three specific laboratory procedures available to physicians. The first is the blood culture, which, as indicated in Chart 1, may be expected to be positive in upwards of 85 per cent of cases during the first week of illness. The second is stool cultures in which the percentage of cases yielding positive cultures progressively increases during the first two weeks of illness. During the first week, however, stool cultures are definitely inferior to blood cultures. The third procedure consists of the demonstration of specific agglutinins in the blood serum of the patient. It will be noted that

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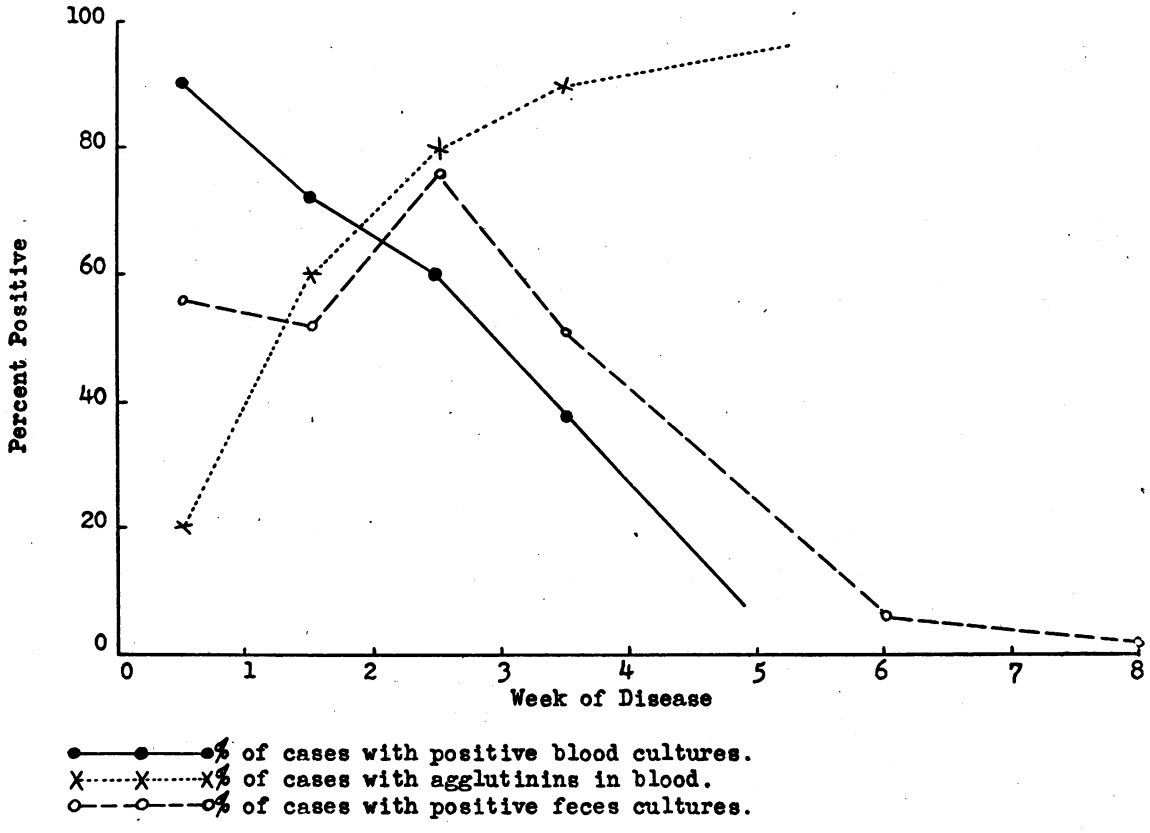

Source: Adapted from Topley and Wilson (2d Ed.; 1941), Fig. 259, P 1198.

Chart 1.—Relation of Isolation of Organisms from Blood and Feces and Agglutination Titer in Cases of Typhoid Fever.

agglutinins begin to appear by the end of the first week of illness but that it is the third week before the percentage of cases positive equals the percentage that yield positive blood cultures during the first week of illness. This time-relationship between blood culture, stool culture and serum agglutinin is shown in Chart 1.

Culturing the blood for *E. typhosa* is a simple procedure that can be readily done by any clinical, hospital or public health laboratory. However, many hospital and clinical laboratories lack adequate materials for efficient examination. One of the most frequent errors is the securing of too small a quantity of blood. A good rule is to obtain at least 20 cc. for the culture.

The recovering of the organisms from stool specimens is a more exacting procedure, and not infrequently laboratory personnel not accustomed to the preparation and use of differential culture media used for such isolations will fail to achieve recoveries from positive stool specimens. Local and state public health laboratory services, where workers in general have more experience with this specific procedure, are available to physicians, hospitals and clinical laboratories to assist in such studies.

Agglutinin tests can be done in any well established hospital, clinical or public health laboratory. Satisfactory antigen suspensions for such tests are available through commercial supply houses or from the state laboratory. For epidemiological purposes, the State Department of Public Health requires that cultures of all isolations of *E. typhosa* be forwarded to the state laboratory for phage typing. By this procedure it is possible to separate *E. typhosa* into some eight specific types which appear to be characteristic and constant for any given strain of organism.* This procedure is frequently of much value in determining the source of any given outbreak of typhoid.

All that has been said with reference to laboratory procedures designed to specifically identify typhoid infections applies almost equally well to infections with *S. paratyphosa A* and *B*.

It should also be noted that any one of a large group of organisms of the genus Salmonella may be responsible for acute gastro-intestinal infections in man. These are usually diagnosed as food poisoning by the physician. They are, however, true infections; the symptoms, as far as is known, are due to the infection and not from the ingestion of preformed toxins. Blood cultures are usually negative and specific diagnosis usually rests upon recovery of the specific organisms from stool cultures. In severe infections, there is a rise in the agglutinin

*Recent work by Craigie and Felix has justified the expansion of this scheme to approximately 24 types which according to preliminary work permits a specific typing of 95 per cent of all cultures of *E. typhosa*. Heretofore only 70 per cent could be typed.

titer in the blood stream. However, since the infections are frequently of short duration and transitory, agglutinin determinations are usually of no value in establishing a specific diagnosis. The state laboratory maintains a Salmonella Typing Service for specific identification of any cultures of Salmonella that may be isolated from such cases. From such studies, valuable information is being obtained relative to the occurrence and distribution of the various Salmonella species in California.

BACILLARY DYSENTERY

In California, bacillary dysentery is currently more prevalent than is typhoid fever. There have been 1,669 reported cases in the past five years. This certainly represents the minimum number of cases actually occurring. The true number is undoubtedly far above this minimum. In bacillary dysentery, diagnosis from the laboratory standpoint rests upon the isolation of the specific organisms from rectal swab or fresh stool specimen cultures. Such cultures are almost constantly positive during the acute phases of the disease if correct laboratory procedures are employed. Blood cultures are rarely positive and serum agglutinins appear inconstantly. The many serological cross reactions with other groups of organisms makes the serum agglutinin of little value.

Again the state laboratory has established a typing service which is available for specific identification of species of these organisms. Because of the epidemiological importance of knowing the specific species occurring in the state it is requested that all such cultures be transmitted to the state laboratory.

TULAREMIA

Thirty-six cases of tularemia have been reported in California during the past five years. In this disease, blood cultures early in the course of the disease and demonstration of rise in serum agglutinins after the second week are of value in establishing specific diagnosis. Positive cultures may also be obtained directly from lesions or buboes. These laboratory services are available in all well established clinical, hospital and public health laboratories.

PLAGUE

Plague still occurs in California, although rarely. In recent years only the bubonic form has been encountered, three cases having occurred during the past five years. Direct examination of smears prepared from aspirated material from the buboe with the application of special staining techniques usually demonstrates the typical bipolar staining organisms. Confirmation is secured by culture and animal inoculation. Due to the danger of handling these organisms in the laboratory the regulations of the State Board of Public Health require that the physician or laboratory communicate by telephone or wire immediately with the state laboratory for specific instructions in the handling of material from suspected cases of plague.

BRUCELLOSIS

During the past five years, the number of cases of brucellosis reported has varied from 260 to 320 per year with a total of 1,391 cases. Evidence indicates that this is probably much below the actual occurrence. The laboratory not infrequently provides all too little aid to physicians in the diagnosis of this disease. The only completely definitive laboratory procedure is the isolation of the specific organism from the blood. Of almost equal significance is the demonstration of rise of serum agglutinin titer during the course of the disease, provided there has been neither administration of vaccine nor performance of skin tests. Either of the latter procedures invalidates the results of serum agglutinin tests. In this disease, repeated blood cultures should be made and repeated agglutinin tests performed. The latter are frequently negative during the quiescent stages of the infection only to become positive during acute exacerbations of the disease. Such a rising agglutinin titer provides good evidence of infection, provided, again, there have been no skin tests nor vaccine administration. In this as in other diseases it should be recognized that the presence of serum agglutinins does not necessarily mean current infection. It means either current or past contact with the specific antigen. On the other hand, infection rarely occurs without agglutinins appearing some time at least during the course of the disease.

RELAPSING FEVER

During the past five years, 60 cases of relapsing fever have been reported in California. This is a summer disease contracted for the most part by vacationists in the Sierra Nevada ranges, known cases having been reported from Big Bear Lake in San Bernardino County and from Lake Tahoe south to Sequoia National Park in the central Sierra Range. There is reason to believe that many additional cases occurred which were not diagnosed or not reported. The laboratory methods that establish the diagnosis are the finding of spirochetes of relapsing fever in stained blood smears of the patient or in blood of mice following inoculation with the patient's blood. This service is available in the state laboratory and the larger local public health laboratories and is provided by some of the larger hospital and clinical laboratories.

RICKETTSIAL DISEASES

In California there occur at least three diseases caused by rickettsiae that present diagnostic problems. These are Rocky Mountain spotted fever, which occurs occasionally in the northern Sierra Nevada Mountain range area; endemic typhus, which appears to be limited to the southern California area south of the Tehachapi Mountains; and "Q" fever, which has been identified within the past year and the total distribution of which is unknown. During the past year it has been most prevalent in the Los Angeles area. In all three of these diseases

the definitive diagnosis rests upon either recovery of the rickettsia from the blood taken in the early stages of the disease or a demonstration of rise in complement fixing antibodies as the disease progresses. The latter specific procedure is now largely replacing the long-used, less specific Weil-Felix reaction which rests on a rise in agglutinin titer to certain strains of proteus organisms. Also it should be noted that *Coxiella burneti,* the causative agent of "Q" fever, does not produce agglutinins to "X" strains of proteus. The recovery and identification of the specific rickettsial agent is a time-consuming and costly procedure not without danger of infection to the laboratory worker. However, the complement fixation tests can be readily carried out. Blood should be collected as near the onset of the disease as possible and again a week to ten days later so that the two tests may be run in parallel in the laboratory. A rise in specific antibody is encountered only in the presence of the specific disease. The presence of complement fixing antibodies in a single blood specimen may mean present or previous infection. At present, as far as the author knows, these diagnostic services are available in California only in the state laboratory.

VIRAL DISEASES

There are a number of diseases caused by viruses in which the laboratory can now aid the physician in arriving at a specific diagnosis. Just as in diseases due to bacteria, the laboratory may recover and identify the specific virus or it may demonstrate rise in specific antibodies to the virus in the serum of the patient. The latter, as in rickettsial diseases, is the more simple and practical procedure. The rise in specific antibodies is demonstrated in various ways. One is by the complement fixation test using a specific viral agent as antigen; a second is by the demonstration of virus neutralizing antibodies by observing the effects of properly balanced virus serum mixtures in laboratory animals.

Some indirect procedures have also been developed such as the chicken cell agglutination inhibition reaction in influenza and the occurrence of cold agglutinins in virus pneumonia. Currently such routine diagnostic services in California are offered in the state laboratory. Certain of the tests are also available in a few hospital and clinical laboratories. Such services include tests for influenza, types A and B; virus pneumonia; psittacosis; the various encephalitides due to either Western equine or St. Louis viruses, and encephalitis due to the mumps virus. In addition, complement fixation tests are available for lymphogranuloma venereum and lymphocytic choriomeningitis. In all of these tests the physician should send in both an acute and convalescent blood specimen, the first taken at the earliest possible moment in the course of the infection and the second, ten days to two weeks later.

During the past several years, the State Viral and Rickettsial Disease Laboratory has been studying and developing diagnostic test procedures. As the program during this formative period was largely investigative there were frequently long delays in getting reports back to physicians. Now that a number of the procedures have become routine tests involving complement fixation, results can be reported within a day or two after the second specimen is received. The laboratory diagnostic service available for a number of the viral and rickettsial diseases is therefore now comparable to the agglutination test service available for the diagnosis of typhoid fever. As the various procedures in the viral and rickettsial field are perfected and simplified diagnostic tests are achieved, the services in this field will undoubtedly come to physicians in their own communities through well equipped hospital, clinical and public health laboratories. This will be the case with the more common viral and rickettsial infections; but with rarer infections such as Rocky Mountain spotted fever, psittacosis and the various encephalitides it will probably not be economical for more than one or a limited number of the laboratories in the state to provide such services to the physicians.

SUMMARY AND CONCLUSIONS

It will be seen from the above discussion that the cardinal principles for the clinicians to keep in mind in the diagnosis of communicable or infectious diseases are that an attempt should be made either to recover and identify the specific organism or to provide the laboratory with an acute and convalescent blood specimen so that rise in titer of specific antibodies may be demonstrated. Either of these two fundamental procedures will provide the physician with a definitive diagnosis by the laboratory. As indicated, since a number of these diseases occur so infrequently and the laboratory techniques are new and not readily available, particularly for the viral and rickettsial diseases, the physician must depend for the time being upon the state laboratory for such service. However, as pointed out with the various diseases, in a number of instances services are available through the usual clinical and hospital laboratory channels upon which the physician depends. The logical procedure for the physician to follow is to secure all available services from the laboratory ordinarily serving him, call upon the local public health laboratory for services not provided by his clinical and hospital laboratory, and refer only those specimens to the state laboratory for which no local services are available. At present, the latter are almost entirely limited to viral and rickettsial disease specimens. As this field of laboratory diagnostic tests develops, undoubtedly more and more laboratories will be able to offer diagnostic service in this field.

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